



Pathophysiology Of *Klebsiella.Pneumoniae* Infections & Prevalence Of Uropathogenic *Klebsiella* In Pregnant Women.

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Abstract: Urinary tract infection (UTI) is one of the most common health problems among pregnant women and the reason for morbidity during pregnancy in worldwide. Occurrence of Gram-negative bacteria is more frequent and next to *E.coli*, *Klebsiella spp.* is a recurrently isolated organism causing UTI. *Klebsiella pneumoniae*, Gram-negative, encapsulated, and nonmobile bacteria are bacteria that normally live in intestines and feces. They also have a high tendency to become antibiotic resistant. A total 183 samples having UTI obtained out of 300 samples from pregnant women, investigated for identification and characterization of the uropathogenic isolates. *Klebsiella pneumoniae* was obtained as the 2nd most bacterial pathogen having 12.5 % of occurrence. The identified pathogens were then screened for pathogenic factors namely haemolysin production, Mannose Resistant and Mannose sensitive Haemagglutination (MRHA, MSHA), Cell surface hydrophobicity and Serum resistance, biofilm formation, production of siderophores by recommended methods. Piperacillin/Tazobactam(P/T) and Norfloxacin (Nx) were found to be the most effective drug against *Klebsiella spp.* and biofilm production (65.2%) was observed as the most frequent pathogenic factor in *Klebsiella spp.*

Key Words: Urinary tract infection, *Klebsiella* spp., Pathogenesis, Pathogenic factors.

1. INTRODUCTION

UTI account for about 10% of primary care consultations by pregnant women and it was reported that up to 15% of women will have one episode of UTI at some time during their life. (Delzell *et al.*,2000) The incidence of UTI reported among pregnant mothers is about 8% ((Delzell *et al.*,2000; Orenstein *et al.*,1999). ‘Anatomically UTI can be classified into lower urinary tract infection involving the bladder and urethra and upper urinary tract infection involving the kidney and pelvis ureter. The majority of the UTI occur due to ascending infection’ ((Delzell *et al.*, 2000; Orenstein *et al.*,1999).This is a common nosocomial pathogen causing nosocomial pneumonia, urinary tract infections, and intra-abdominal infections. They are widely distributed in nature, occurring as commensals in animal and human intestines and also as saprophytes in soil. When it gets into other areas of the body, however, it leads to illnesses like pneumonia, bloodstream infections, meningitis, and urinary tract infections. Most cases of *K. pneumoniae* infection occur in a hospital setting. *Klebsiellae* are ubiquitous in nature.

The genus *Klebsiella* consists of Gram negative, non-sporing, capsulated, non-motile bacilli which grow well on ordinary media and produce pink mucoid colonies on MacConkey’s agar. This is a nosocomial pathogen which becomes the cause of urinary tract Infections, nosocomial pneumonia and intra-abdominal infections. The classification of *Klebsiella* has undergone various modifications. They have been classified into two species namely *K. pneumonia*, and *K. oxytocea*. *K. pneumonia* is further subdivided into four subspecies, namely, aerogenes, pneumonia, ozaenae and rhinoscleromatis, unlike other subspecies, *K. oxytocea* is indole positive (Baveja, 2005).

Klebsiella isolates are short, plump bacilli, which are about 1-2 μm x 0.5- 0.8 μm in size. “They grow well on ordinary media at optimum temperature of 37°C in 18-24 hours. On MacConkey’s agar, there are appearance of large, mucoid and pink to red coloured colonies. Mucoid nature of colonies is due to capsular material produced by the organism. Fermentations of sugars (glucose, lactose, sucrose, mannitol) occur with production of acid and gas. Biochemical characteristics represent that they are urease positive, indole negative, MR negative, VP positive. and citrate positive (IMVic - - + +). These reactions are typical of *K. pneumonia* subsp. Aerogenes” (Baveja, 2005).

It is described in a study in 2006, nosocomial acquired bacteremia tends to associate most commonly with neoplastic conditions, as well as having a mortality rate more than twice that of community-acquired bacteremia. Furthermore, 33.3% of the nosocomial isolates demonstrated resistance to cephalosporin, and 22% to ciprofloxacin. Invasive medical procedures increased the likelihood of nosocomial infection, and previous usage of cephalosporin and ciprofloxacin increased the chances of resistance development. In addition, diabetes mellitus and chronic liver diseases were most commonly associated with community acquired

bacteremia. These isolates displayed lower levels of resistance to cephalosporin and ciprofloxacin (3.7% and 4.2% respectively) (Kang *et al.*, 2006).

Pathophysiology-

K. pneumoniae is a Gram-negative bacterium, non-motile, encapsulated, facultative anaerobic, rod-shaped bacterium which measures 2 µm by 0.5 µm. It is also found in the urine and forms the origin of some urinary tract infections acquired in hospitals (nosocomial infection), particularly those engaged in risk services such as surgical services, intensive care or long stays. It could be due to inadequate cleaning of the hands of hospital staff, poor sterilization of objects used and also the use of broad spectrum antibiotics and an altered anatomical barrier which in turn may be due to extensive burns or therapeutic interventions (tracheotomy, intubation, urinary catheter, intravenous catheter, prosthesis etc.) (WenChien *et al.*, 2002). It can be seen in many hospital cases around the world.

The signs and symptoms of *Klebsiella* infection depend on the location of infection. General signs of infection might include fever, chills, redness, swelling, pain, and drainage or pus from a wound or surgical site.

As such, further studies of the strains were the need of the hour and also undertaken. In 2004, the bacterium was isolated and sequenced from a patient. *K. pneumoniae* is commonly found in the gastrointestinal tract and also in the hands of hospital personnel (Podschun *et al.*, 1998). However, “it is pertinent to mention that the rates of infections caused by community-acquired *K. pneumoniae*, are not the same among world populations; they vary considerably” (Wenchien *et al.*, 2002). Generally, infections or diseases are either nosocomial or acquired from the hospital. According to Podschun *et al.* (1998) around “eight percent (8%) of nosocomial bacterial infections in the United States and in Europe were caused by *Klebsiella pneumoniae* and *Klebsiella oxytca* and the diseases that followed were urinary tract infections, pneumonia, septicemias, and soft tissue infections” (Podschun *et al.*, 1998). The diseases caused by *Klebsiella pneumoniae* can be very fatal and result in death of the patients having lack of immunity. Differences in the disease severity may be affected by different virulence factors.

The bacterial pathogens overcome innate host immunity through several means. They possess a polysaccharide capsule, which is the main determinant of their pathogenicity. The capsule is composed of complex acidic polysaccharides. Its massive layer protects the bacterium from phagocytosis by polymorphonuclear granulocytes. In addition, the capsule prevents bacterial death caused by bactericidal serum factors. The bacteria also produce multiple adhesins. These may be fimbrial or nonfimbrial, each with

distinct receptor specificity. These help the microorganism adhere to host cells, which is critical to the infectious process.

Availability of iron increases host susceptibility to *K. pneumoniae* infection. Bacteria are able to compete effectively for iron bound to host proteins because of the secretion of high-affinity, low molecular weight iron chelators known as siderophores. This is necessary because most host iron is bound to intracellular and extracellular proteins. In order to deprive bacteria of iron, the host also secretes iron-binding proteins.

K. pneumoniae utilizes a variety of virulence factors, especially capsule polysaccharide, lipopolysaccharide, fimbriae, outer membrane proteins and determinants for iron acquisition and nitrogen source utilization, for survival and immune evasion during infection. This article aims to present the prevalence of the pathogenesis of *K. pneumoniae* causing UTI in pregnancy.

Aims and objectives:-

1. Isolation and identification of uropathogenic *Klebsiella* in pregnant women.
2. Antimicrobial sensitivity pattern of the isolated *Klebsiella*.
3. Study of the pathogenic factors in the *Klebsiella* isolates.

2.MATERIAL AND METHODS

A research study was conducted on 300 midstream urine samples obtained by informed consent of the pregnant women who were suspected to have UTI, attending different antenatal clinics at Bhubaneswar and Puri. The pregnant women who were on antibiotic therapy within last two weeks were not involved in the study. After identification of the uropathogenic isolates a total of 183 cases were found positive for UTI.

2.1. Processing for the *Klebsiella* Isolates:-

The samples were processed by standard microbiological techniques (Collee *et al.*, 1996). Then the identification and characterization of isolated bacteria was done by microscopic examination, colony morphology on blood agar and Mac-conkey's agar, gram staining followed by standard biochemical tests according to Cheesbrough (2002,2004) and Bergey's Manual of Systematic Bacteriology.

2.2. Testing of Antibiotic sensitivity pattern:-

The isolates were subjected to antibiotic susceptibility testing using Kirby-Bauer disc diffusion method (Collee *et al.*, 1996), which was done on Mueller Hinton Agar plate by using the different antimicrobial agents, as per the CLSI guidelines. The antibiotics discs were inserted by help of a sterile forceps and then they were gently pressed down to make sure the complete and uniform contact of the disc to agar surface. Discs of the following antimicrobial agents were put up as follows - Amikacin (AK) (30µg), ampicillin/Sulbactam (A/S) (20 µg), ceftazidime

(CAZ) (30 µg), cefotaxime(CTX) (30 µg), cefuroxime (CXM) (30 µg), ciprofloxacin(CIP) (5 µg), gentamicin(GEN) (30 µg), imipenem (IPM) (10 µg), piperacillin-tazobactam (PIT) (110 µg), nitrofurantoin(NIT) (300 µg), norfloxacin(NX) (5 µg). Then the plates were incubated within 15 minutes of discs application, in the incubator at 37°C for 24 hours. The zone of inhibition produced by diffusion of drug from disc into the surrounding medium was measured to find out the degree of sensitivity.

2.3. Methods for study of the uropathogenic factors of the isolates:-

The colonies identified as *Klebsiella* were screened for the study of pathogenic factors namely haemolysin, Mannose Resistant and Mannose Sensitive Haemagglutination (MRHA, MSHA), Cell surface hydrophobicity and Serum resistance, by recommended methods (Sharma *et al.*, 2007; Vagarali *et al.*, 2008).

- **Haemolysin production** was detected by determining the presence of a zone of complete lysis of erythrocytes around the colony and clearing of the medium (5% sheep blood agar).
- Detection of **haemagglutination (HA)** was studied by clumping of erythrocytes by fimbriae of bacteria in the presence of D-mannose.
- **Cell surface hydrophobicity** property of the isolates was tested by salt aggregation test by using different molar concentration of ammonium sulphate.
- For the **serum resistance** test, overnight culture of organism on blood agar plates was suspended in Hank's balanced salt solution. Equal volume of this bacterial suspension and serum (0.05 ML) were incubated at 37°C for 3h. The Resistance of bacteria to serum bactericidal activity was determined by the percentage of bacteria surviving after 180 minutes of incubation with serum in relation to the original count.
- Production **Gelatinase** was tested using gelatin agar plates inoculated with organism and incubated at 37°C for 24 h. The plate was then flooded with 1% tannic acid solution. Developments of opacity around colonies were considered as positive for gelatinase.
- **Biofilm formation** was assessed by Tissue Culture Plate method (tcp) i.e. 'TCP assay'. Optical densities (OD) of stained adherent bacterial cells forming biofilms on all sides of the wells of tissue culture plates, were determined with a micro ELISA auto reader at wavelength of 620nm and were graded as per Christensen *et al.* (1985). These OD values were considered as an index of bacteria adhering to surface and forming biofilms. (Tolle *et al.*, 1998)
- The test for **Siderophore production** was done by using chrome azurol sulfonate (CAS) agar diffusion assay, in which CAS detects colour change of CAS-Iron complex from blue to orange halo, which was taken as positive after chelation of the bound iron by siderophores.

3. RESULT

After identification and characterization of the uropathogenic isolates, Out of the 183 samples having UTI, *Klebsiella pneumoniae* was obtained as the 2nd most bacterial pathogen. It was found that, occurrence of Gram-negative bacteria was more frequent and *Klebsiella spp.* was a recurrently isolated organism having (23)12.5 % of occurrence.

Table-3.1:- Antibiotic sensitivity and resistant pattern of *Klebsiella spp.*

Antibiotics	Sensitive (%)	Intermediate sensitive (%)	Resistant (%)
Amikacin (Ak)	18 (78.26%)	0	5 (21.73%)
Ampicillin/sulbactam (A/S)	12 (52.17%)	0	11 (47.82%)
Ceftazidime (Caz)	9 (39.13%)	1 (4.34%)	13 (56.52%)
Cefotaxime(Ctx)	7 (30.43%)	3 (13.04%)	13 (56.52%)
Cefuroxime (Cxm)	14 (60.86%)	1 (4.34%)	8 (34.78%)
Ciprofloxacin (Cip)	18 (78.26%)	2 (8.69%)	3 (13.04%)
Gentamycin(G)	16 (69.56%)	0	7 (30.43%)
Imipenem (I)	17 (73.91%)	1 (4.34%)	5 (21.73%)
Norfloxacin (Nx)	19 (82.60%)	0	4 (17.39%)
Nitrofurantoin(Nit)	17 (73.91%)	1 (4.34%)	5 (21.73%)
Piperacillin/Tazobactam(P/T)	19 (82.60%)	1 (4.34%)	3 (13.04%)

The antibiotic sensitivity study (**Table-3.1**) of all the 23 *Klebsiella* isolates revealed that Piperacillin/Tazobactam(P/T) and Norfloxacin (Nx) were the most effective drug to which 85.6% of isolates were sensitive followed by amikacin (Ak) and Ciprofloxacin (Cip) to which 78.26% of the isolates were sensitive. Cefotaxime(Ctx) and Ceftazidime (Caz) were found as the least effective drug showing to which maximum isolates showed resistance at a frequency rate of 56.52% each.

Table-3.2:- Detection of various pathogenic factors in the *Klebsiella* isolates.

Pathogenic markers	No. positive (%) <i>Klebsiella</i> spp.(N=23)
Hemolysin production	0
Haemagglutination (MRHA & MSHA)	11 (47.8)
C.S.H (Cell Surface Hydrophobicity)	8(34.7)
Serum resistance	14(60.8)
Gelatinase test	13(56.5)
Siderephore production assay	1(4.3)
Biofilm formation	15(65.2)

The presence of pathogenic factors of the *Klebsiella* isolates were assessed and the data presented in Table no. 3.2 The results showed that hemolysin production was completely absent in *Klebsiella* spp. whereas, the most frequent pathogenic factors in *Klebsiella* was biofilm production (65.2%) followed by serum resistance (60.8%). Production of Siderephore was observed as the least frequent pathogenic factor in *Klebsiella* spp.

4. DISCUSSION

Antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms. The resistance to the antimicrobials has increased over the years. Resistance rates vary from country to country (Kahan *et al.*, 2006; Sharma *et al.*, 2005). There is an evidence for increase in antibiotic resistance In Pattukkottai. In most Indian studies *Klebsiella* spp. occupy second place among uropathogens. However, in the present study this was also consistent occupying the the second (12.5 %) common uropathoges. As a whole *Klebsiella* spp. in our study area appeared to be highly susceptible to Piperacillin/Tazobactam(P/T), Norfloxacin (Nx) amikacin (Ak), ciprofloxacin (Cip), and nitrofurantoin (Nit), which are the common antibiotics prescribed for UTI. As suggested by Johnson, (2000) even though there is no clinical role for study of pathogenic markers, occurrence of multiple phenotypically positive virulence markers for single strains denotes increased virulence and thereby leads to more complications (Schembri *et al.*, 2005). Pathogenic markers for *Klebsiella pneumoniae* can be identified by presence of mucoid colonies in culture media and it revealed that 4.3% of the isolates showed positivity during the studies, identified that mucoid colonies have the ability of producing aerobactin and siderophore which helps in the pathogenesis, whereas maximum isolates of *Klebsiella* spp. (65.2%) were biofilm producers.

5. CONCLUSION

This study has revealed that screening for bacteriuria during pregnancy is a useful investigation and the examination of bacteriuria with detection of pathogenic factors, which may help in the prevention of complications associated with UTI in pregnant women. Justified use of antibiotics is important to limit the emergence and spread of antibiotic resistance in bacteria. The present study will help in an appropriate therapy of infection caused by *Klebsiella* species in pregnant women.

More study of pathogenic factors and cultures of the uropathogenic *Klebsiella* will be advocated for appropriately management of UTI.

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